ITALIAN JOURNAL OF ANIMAL SCIENCE, 2017
VOL. 16, NO. 4, 546–551
http://dx.doi.org/10.1080/1828051X.2017.1292830

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PAPER

OPEN ACCESS

Response of broiler chickens to dietary inclusion of fermented canola meal under heat stress condition

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ABSTRACT

Feeding high levels of canola meal to monogastric animal require reducing antinutritional factors such as glucosinolates and fibre. Solid state fermentation is known to reduce antinutritional factors and improve nutritional quality of feedstuffs. In this study, canola meal was treated with *Lactobacillus salivarius* in solid state fermentation for 30 days and included in diet with 4 levels of 0 (control), 10, 20, and 30%. From 29 to 35 days of age, equal number of birds from each dietary treatment was exposed to either 23 ± 1 °C (unheated) or 36 ± 1 °C (heated). Results showed that irrespective of temperature, weight gain (WG) and feed conversion ratios (FCR) were not affected by inclusion of fermented canola meal (FCM). Diet also did not affect carcass yield, plasma triiodothyronine (T_3) and tetraiodothyronine (T_4), and body temperature. As expected, heated birds had lower carcass yield and T_3 than their unheated counterparts. In conclusion, although dietary inclusion of FCM at levels more than 10% retarded growth performance during 1 to 28 days of age, no detrimental effects on performance was observed when FCM included up to 30% during 29 to 35 days of age under both unheated and heated conditions.

ARTICLE HISTORY
Received 17 November 2016
Revised 5 February 2017
Accepted 6 February 2017

KEYWORDS
Fermentation; rapeseed meal; broilers; heat stress; thyroid

Introduction

Despite availability of double zero, low-glucosinolates, and low-erucic acid canola meal (CM), its usage in monogastric animal feeding is still limited due to antinutritional factors such as tannins, phytic acid, sinapine, glucosinolates and fibre (Kocher et al. 2000). These antinutritional factors may reduce feed intake, impair growth performance, decrease dietary protein and energy digestibility (Meng & Slominski 2005), and cause abnormalities of thyroid function in chickens (Tripathi & Mishra 2007; Woyengo et al. 2011). Among the available approaches to reduce the antinutritional factors of CM, solid state fermentation (SSF) is the most promising (Al-Asheh & Duvnjak 1995; Aljuobori et al. 2014; Niu et al. 2015; Croat et al. 2016). Fermentation of rapeseed meal with a combination of *Bacillus subtilis, Candida utilis* and *Enterococcus faecalis* at 30 °C for 3 days resulted in degradation of 96% of glucosinolates, 33% of crude fibre, 20% of phytic acid, and 36% of tannin (Hu et al. 2016). Study by Chiang et al. (2010) showed that feeding rapeseed meal fermented with a combination of *Lactobacillus fermentum, Enterococcus faecium, Saccharomyces cerevisae* and *Bacillus subtilis* at 30 °C for 3 days, improved growth performance and intestinal morphology of broilers compared to those fed unfermented rapeseed meal. Previous study in our laboratory using *Lactobacillus salivarius* also indicated significant improvement in nutrient composition of fermented CM (Aljuobori et al. 2014).

The detrimental effects of heat stress on growth performance and feed intake is well known in broiler chickens (Howlider & Rose 1987; Geraert et al. 1996). To overcome, at least partially, the adverse effects of heat stress, it is recommended to use nutrient concentrated diet and limit the use of fibrous dietary ingredients. High dietary fibre may reduce the availability of essential amino acids (Koelkebeck et al. 1998). To the best of the authors' knowledge, there are no published data on use of fermented CM for chicken reared under heat stress condition. Therefore, the present study aimed to evaluate the effect of feeding fermented canola meal (FCM) on growth performance, and plasma concentrations of triiodothyronine (T_3) and

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tetraiodothyronine (T_4) in broiler chickens reared under heat stress condition.

Materials and methods

Fermentation procedure

Lactobacillus salivarius (GenBank accession number: KF303794) which has been isolated from Malaysian fermented soybean (tempeh) was incubated in MRS broth (Merck, Germany) for 24 h at 37 °C as described previously (Aljuobori et al. 2014). After incubation, the cultures were centrifuged at 10,000*g* for 10 min at 4 °C. The supernatants were discarded and cell pellets were freeze-dried. About 200 g of freeze dried *L. salivarius* were prepared and stored at −20 °C for CM treatment. Canola meal was obtained from a commercial feed mill in United Arab Emirates. About 200 kg of CM were fermented by *L. salivarius* as explained previously (Aljuobori et al. 2014). Control CM was prepared at the same time without inoculant addition. At the end of the fermentation period, samples of the untreated and inoculated CM were dried at 50 °C for 5 days.

Chemical analyses

The samples of CM and FCM were finely ground using a coffee grinder (Panasonic, Malaysia) and dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash were determined according to AOAC methods 925.09, 988.05, 920.39, 978.10 and 942.05, respectively. Total glucosinolates was determined based on alkaline degradation and subsequent reaction of released 1-thioglucose with ferricyanide (Jezek et al. 1999; Gallaher et al. 2012). Amino acid concentrations were determined as previously indicated (Soleimani et al. 2010).

Birds and housing

All experimental procedures were conducted in accordance with Universiti Putra Malaysia Research Policy on Animal Care. A total of 200 day-old male broiler chicks (Cobb 500) were obtained from a commercial hatchery. Upon arrival (day 1) the chicks were weighed and randomly assigned in groups of 5 to 40 battery cages with wire floors in two identical and adjacent environmentally controlled rooms. The chicks were raised at 32 ± 1 °C, and then the temperature was gradually decreased until 24 ± 1 °C was reached by day 21. Water and feed were available at all times and continuous fluorescent lighting was provided.

Experimental diets

Isonitrogenous and isocaloric starter (day 1 to 21) and grower (day 22 to 35) diets (Table 1) containing 0, 10, 20 and 30% FCM were provided.

Heat challenge and data collection

From day 29 to 35, equal numbers of chickens from each dietary group (5 cages of birds) were subjected to either 23 ± 1°C (unheated) or 36 ± 1 °C (heated) throughout. The mean relative humidity was 70–80% and 65–75% for unheated and heated conditions, respectively. On day 35, two birds from each cage were randomly selected and rectal temperature (BT) was measured using an electronic thermometer. The probe was inserted about 1–1.5 cm into the rectum for about 30 s until a fixed reading was obtained. Following recoding of body temperature, blood samples (3 mL) were collected from the wing vein using heparinised tubes. The samples were centrifuged at 2500*g*, for 10 min and plasma were obtained and stored at −20 °C until being used for hormone analysis. Plasma thyroid hormone concentration was determined by 125 I labelled RIA kits for T_3 (IM1699, Immunotech, Czech Republic) and T_4 (IM1447 Immunotech, Czech Republic) (Okuliarova et al. 2011). After blood sampling, the birds were slaughtered according to halal method (Farouk et al. 2014) and processed to determine hot carcass (excluding giblets, % of live weight) and breast and leg (drumstick with thigh) weight (% of carcass) (Mushtaq et al. 2005).

Statistical analysis

Data were analysed with the aid of SAS software package (V 9.1, SAS Institute Inc., Cary, NC). One-way ANOVA was used to analyse data from day 1 to 28. Data from day 29 to 35 were analysed using diet, temperature and their interactions as main effects. When interactions between main effects were significant, comparisons were made within each experimental variable. Differences between means were analysed by Duncan's multiple range test. Statistical significance was considered at $p \leq .05$.

Results

Data for chemical analyses of CM and FCM were also used by Aljuobori et al. (2014) in an earlier study. The pH values determined at the initial and end stage of fermentation by *L. salivarius* were 5.7 and 4, respectively, while the colony forming units were 2 × 109 and

Table 1. Composition of experimental diets.

	FCM[a] inclusion rate, %							
	Starter (1 to 21 d)				Grower (22 to 35 d)			
	0	10	20	30	0	10	20	30
Ingredient, %								
Maize	50.2	47.75	45.26	42.83	57.99	55.89	53.1	50.66
Soybean meal	36.26	28.16	19.98	11.79	26.68	18.82	10.31	2.12
Fermented canola meal	0.00	10.00	20.00	30.00	0.00	10.00	20.00	30.00
Maize gluten	5.00	5.00	5.00	5.00	6.00	6.00	6.00	6.00
Palm oil	3.94	4.65	5.33	6.01	5.02	5.23	6.38	7.06
Dicalcium phosphate	1.88	1.79	1.70	1.61	1.61	1.25	1.43	1.34
Limestone	1.20	1.16	1.12	1.08	1.17	1.28	1.09	1.05
Premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.14	0.11	0.10	0.08	0.03	0.02	0.01	0.00
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Sodium bicarbonate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-lysine Hcl	0.0	0.0	0.13	0.22	0.12	0.13	0.30	0.39
Calculated composition								
ME, kcal/kg	3050	3050	3050	3050	3200	3200	3200	3200
Crude protein, %	23	23	23	23	20	20	20	20
Calcium	1.0	1.0	1.0	1.0	0.9	0.9	0.9	0.9
Nonphytate phosphorous	0.45	0.45	0.45	0.45	0.39	0.35	0.39	0.39
Crude fibre	3.45	3.79	4.1	4.47	3.07	3.45	3.75	4.09
Digestible lysine	1.02	0.92	0.96	0.96	0.93	0.86	0.92	0.92
Digestible methionine + cysteine	0.80	0.79	0.80	0.80	0.64	0.65	0.66	0.67
Total glucosinolates, μmol/g	0.00	1.36	2.72	4.08	0.00	1.36	2.72	4.08

[a]Fermented canola meal.
[b]Supplied per kilogram of diet: vitamin A, 8000 U; vitamin D_3, 1000 U; vitamin E, 30 U; vitamin K_3, 2.5 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.01 mg; niacin, 30 mg; d-biotin, 0.045 mg; vitamin C, 50 mg; D-pantothenate, 8 mg; folic acid, 0.5 mg; Mn, 70 mg; Fe, 35 mg; Zn, 70 mg; Cu, 8 mg; I, 1 mg; Se, 0.25 mg; Co, 0.2 mg.

Table 2. Effect of dietary inclusion of fermented canola meal (FCM) on feed intake, weight gain (WG) and feed conversion ratios (FCR) of broiler chickens from 1 to 28 days of age.

	FCM inclusion rate, %			
	0	10	20	30
Feed intake, g/bird	2073 ± 27	2043 ± 31	2043 ± 27	2073 ± 28
WG, g/bird	1328 ± 14[a]	1295 ± 25[ab]	1263 ± 18[bc]	1219 ± 20[c]
FCR, feed/gain	1.56 ± 0.01[b]	1.58 ± 0.01[b]	1.61 ± 0.01[b]	1.70 ± 0.03[a]

[a,b,c]Means ±SEM within a row with no common superscripts differ at $p < .05$.

1×105 per g, respectively (see Appendix 1). Fermentation increased CP and decreased CF and total glucosinolates content of FCM as compared to CM (see Appendix 2). However, there were no significant differences between AA content, ash, EE and AME of CM and FCM on DM basis.

The effect of diet on WG, feed intake and FCR during day 1 to 28 are presented in Table 2. Incorporating FCM at 20 and 30% caused a significant reduction in WG when compared to those fed control diet. No significant effect was observed on FCR, when birds were fed diets with 20% FCM. Feed intake was not affected throughout the experimental period. There were no significant temperature × diet interactions for WG, feed intake and FCR (Table 3). Inclusion of FCM up to 30% had no adverse effects on WG, FCR and feed intake from day 29 to 35. The heated chickens had significantly lower feed intake and WG and higher FCR than the unheated counterparts. Neither diet (0% FCM = 4%; 10 FCM = 2%; 20% FCM = 2%; 30% FCM = 6%) nor heat treatment (Unheated = 2%; Heated = 5%) had significant effect on mortality rate.

Table 3. Effects of heat treatment (constant 36 ± 1 °C) and dietary inclusion of fermented canola meal (FCM) on feed intake, weight gain (WG) and feed conversion ratios (FCR) of broiler chickens from 29 to 35 days of age.

	Feed intake, g/bird	WG, g/bird	FCR, feed/gain
Diet			
0	990 ± 56	441 ± 45	2.36 ± 0.14
10	983 ± 66	431 ± 51	2.42 ± 0.15
20	981 ± 72	428 ± 52	2.43 ± 0.14
30	983 ± 58	400 ± 36	2.50 ± 0.12
Temperature			
Heated	809 ± 17[b]	296 ± 9[b]	2.76 ± 0.07[a]
Unheated	1160 ± 16[a]	554 ± 1[a]	2.11 ± 0.04[b]
Analysis of variance		Probabilities	
Diet	0.99	0.37	0.61
Heat	0.0001	0.0001	0.0001
Diet × Temperature	0.36	0.19	0.71

[a,b]Means ±SEM within a column-subgroup with no common superscripts differ at $p < .05$.

Table 4. Effect of heat treatment (constant 36 ± 1 °C) and dietary inclusion of fermented canola meal (FCM) on body temperature (BT) and plasma concentrations of triiodothyronine (T_3), and tetraiodothyronine (T_4) in broiler chickens at 35 days of age.

	BT, °C	T_3, nmol/L	T_4, nmol/L
Diet			
0	43.01 ± 0.35	2.65 ± 0.20	17.02 ± 1.50
10	42.91 ± 0.38	2.51 ± 0.17	19.07 ± 1.10
20	42.74 ± 0.30	2.88 ± 0.18	19.00 ± 1.04
30	42.66 ± 0.43	2.74 ± 0.21	17.10 ± 0.72
Temperature			
Heated	43.84 ± 0.09[a]	2.49 ± 0.11[b]	17.00 ± 0.52
Unheated	41.81 ± 0.12[b]	2.90 ± 0.14[a]	19.10 ± 0.95
Analysis of variance		Probabilities	
Diet	0.37	0.55	0.37
Temperature	0.0001	0.03	0.06
Diet × Temperature	0.11	0.63	0.57

[a,b]Means ±SEM within a column-subgroup with no common superscripts differ at $p < .05$.

Table 5. Effects of heat treatment (constant 36 ± 1 °C) and dietary inclusion of fermented canola meal (FCM) on carcass yield (%) in broiler chickens at 35 days of age.

	Carcass	Breast	Leg*
Diet			
0	72.7 ± 1.1	33.6 ± 1.0	29.5 ± 0.5
10	71.9 ± 0.6	33.5 ± 0.4	28.7 ± 0.4
20	71.1 ± 0.5	34.4 ± 0.7	28.9 ± 0.4
30	71.4 ± 0.5	35.3 ± 0.6	28.8 ± 0.5
Temperature			
Heated	70.5 ± 0.5[b]	34.8 ± 0.5	29.6 ± 0.3[a]
Unheated	73.1 ± 0.3[a]	33.6 ± 0.4	28.3 ± 0.3[b]
Analysis of variance		Probabilities	
Diet	0.35	0.21	0.46
Temperature	0.0001	0.08	0.002
Diet × Temperature	0.92	0.21	0.46

*Drumstick + thigh.
[a,b]Means ±SEM within a column-subgroup with no common superscripts differ at $p < .05$.

There were no significant diet × temperature interactions for T_3, T_4 and BT (Table 4). Diet had no significant effect on T_3, T_4 and BT. Irrespective of dietary groups, the heat treatment resulted in significant elevation of BT. Heated birds had significantly lower T_3 than those in unheated one. No significant changes in T_4 were observed following the heat treatment.

No significant diet × temperature interactions were observed for eviscerated carcass, breast and leg weight (Table 5). Irrespective of temperature there was no effect of diet on carcass characteristics. The unheated birds had significantly greater carcass and lower leg weight than those under heat stress. However, breast weight was not affected by heat treatment.

Discussion

Results from this study confirmed the previous reports beneficial effect of solid state fermentation on nutritional value and nutrient digestibility (Chiang et al. 2010; Aljuobori et al. 2014). Fermentation of CM using *L. salivarius* reduced total glucosinolate and crude fibre content by 38 and 16%, respectively. However, despite the apparent improvement in nutritional value, chickens cannot be fed more than 10% FCM during the starter period without adverse effect on their growth performance. This is corroborated with previous findings that fermented rapeseed meal could only be used up to 10% in the broiler starter diets (Xu et al. 2012). There is a possibility that the reduction in the level of glucosinolates in FCM was insufficient to allow inclusion rates of more than 10%. Glucosinolates content of 10% FCM diet was similar in our study and that of Xu et al. (2012). In our study, diets containing 30, 20 and 10% FCM had 4.08, 2.72 and 1.36 μmol/g of glucosinolates, respectively. Generally, the level of glucosinolates in poultry diets should be less than 2.5 μmol/g to ensure optimum performance (Mushtaq et al. 2007).

It is interesting to note that during 29 to 35 days of age, unheated and heated birds can be fed FCM up to 30% without any detrimental effect on growth performance. It seems that older birds can tolerate higher FCM levels. The phenomenon could be associated with the changes of glucosinolates tolerance by age. Tripathi and Mishra (2007) suggested that younger animals are more sensitive to glucosinolates than older ones. The higher sensitivity of chicks to antinutritional factors of FCM in early stages of life may contributed to their physiological status where they are still in development phase for some physiological functions such as digestion and digestive enzyme activity (Pearson et al. 1983; Nitsan et al. 1991; Mushtaq et al. 2007).

In the present study, irrespective of heat treatment, all birds had similar relative weight of breast, carcass and leg. Similarly, Ahmad et al. (2007) reported that various levels of dietary CM had negligible effect on carcass characteristics, and liver weight in broilers. Regarding the heat effect, we observed reduction in carcass and increase in leg yield, but no effect on breast yield. Similarly, Akşit et al. (2006) showed that heat-stressed chickens had significantly lower carcass but not breast yield. Laganá et al. (2007) reported a higher leg yield in heat exposed chickens similar to our observation. The differential effect of heat treatment on breast and leg muscles could be associated with their energetic characteristics. While breast muscle is mainly glycolytic, leg muscle is oxidative and thus, their respective substrates differ from glucose to fatty acids (Baziz et al. 1996). Similar to our results, previous studies in chickens (Xu et al. 2012) and ducks (Fazhi et al. 2011) demonstrated that FCM inclusion in diet did not affect the thyroid hormones. Schöne et al. (1993) suggested that glucosinolates in CM may destroy cellular T_3 receptors and thus increase the thyroid

hormones level in blood in a feed-back response. Therefore, it could be extrapolated that the level of glucosinolates in FCM in our study was not enough high to induce the T_3 or T_4 reduction considerably. Irrespective of diet, the heat treatment significantly reduced plasma level of T_3.

Conclusions

Although fermentation reduced fibre and glucosinolates content of CM, it is still insufficient to enhance the FCM dietary inclusion to levels more than 10% during the starter phase. However, during day 29 to 35 and under both unheated and heated conditions, broilers can be fed up to 30% FCM without any detrimental effect on performance.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding

The work was funded by the Ministry of Higher Education, Malaysia under the Long-Term Research Grant Scheme (LRGS).

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Appendix 1

Specifications of canola meal (CM) after 30 days of fermentation with *L. salivarius*.

	pH	c.f.u/g CM
Before fermentation	5.7 ± 0.10^{a}	2×10^{9}
After fermentation	4.0 ± 0.15^{b}	1×10^{5}

[a,b]Means within a row with no common superscripts differ at $p < .05$.

Appendix 2

Nutrient composition of canola meal (CM) and fermented canola meal (FCM) (DM basis).

Item, %	CM	FCM	Pooled SEM	*p*-value
Dry matter	91.9	88.8	0.8	.017
ME, kcal/kg*	2446	2420	31.9	.451
Ash	6.2	6.3	0.6	.231
Crude protein	41.2	42.2	0.3	.013
Crude fibre	12.0	10.1	0.5	.033
Ether extract	3.3	3.5	0.4	.641
Glucosinolates, μmol/g	22.0	13.6	1.7	.010
Amino acids				
Aspartic acid	2.57	2.70	0.19	.463
Serine	1.81	1.83	0.07	.771
Glutamic acid	6.59	6.89	0.42	.507
Glycine	2.13	2.15	0.08	.905
Histidine	1.22	1.20	0.03	.597
Arginine	2.54	2.50	0.09	.756
Threonine	1.81	1.83	0.06	.794
Alanine	1.59	1.66	0.10	.550
Proline	2.37	2.45	0.13	.591
Cysteine	1.07	1.04	0.61	.597
Tyrosine	1.03	1.1	0.03	.643
Valine	1.90	1.94	0.10	.663
Methionine	0.77	0.76	0.05	.830
Lysine	1.65	1.72	0.14	.646
Isoleucine	1.35	1.39	0.07	.616
Leucine	2.63	2.71	0.13	.567
Pheylalanine	1.79	1.76	0.05	.636
Tryptophan	0.39	0.39	0.01	.561

*ME: metabolisable energy.